



June 12, 1998

Mr. Kevin Mayer
US EPA Region IX SFD7
75 Hawthorne St.
San Francisco, CA 94105

Dear Mr. Mayer:

I also attended the NGWA Southwest Focused Ground Water Conference in Anaheim, CA on June 3-5, 1998. Since you expressed interest in the perchlorate groundwater problem, I wanted to send you a few documents outlining my company's experience with the biodegradation of perchlorate. Applied Research Associates, Inc. has been developing and implementing biotechnology for perchlorate applications for over 7 years. We have demonstrated success in degrading perchlorate to below detectable limits from brine solutions produced from the regeneration of ion exchange resins. We also designed and installed a prototype system to reduce perchlorate in a brine wastewater. This prototype has continued to perform well since December 1997.

Please take a few moments to review the enclosed documents and call me if you have any questions, comments or interest. If I am not available, please speak with Ed Coppola. Thank you for your time.

Sincerely,



Glen McDonald

Enclosure



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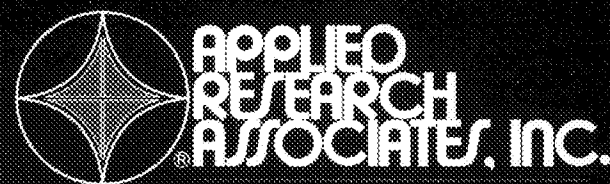
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For answers to your environmental questions and concerns, contact:

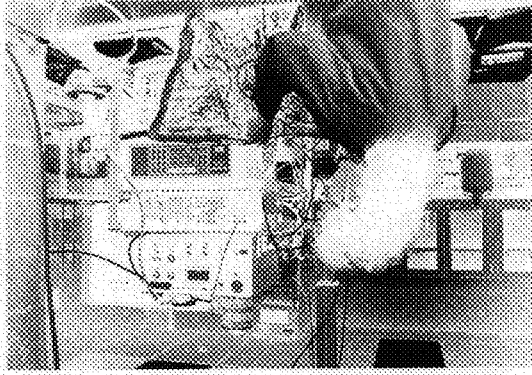


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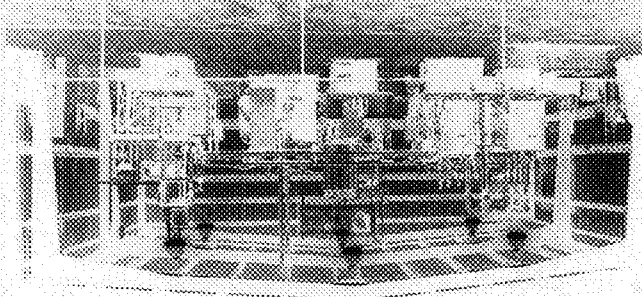
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Environmental Risk Assessment

- ◆ Atmospheric, terrestrial, and aquatic contaminant fate and transport.
- ◆ Atmospheric, terrestrial, and aquatic risk assessment modeling.

Systems for Treating Hazardous Waste



- ◆ Field-scale reactor systems for biodegradation of perchlorate from wastewater.
- ◆ Supercritical water oxidation reactors for industrial wastestreams.

Remediation of Subsurface Contamination

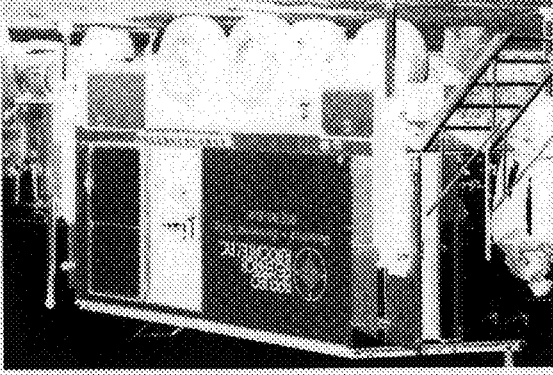
- ◆ Leaders in understanding and applying natural attenuation and in situ biodegradation of petroleum hydrocarbons and chlorinated solvents.
- ◆ Design and performance modeling of:
 - Systems for enhanced removal of chlorinated solvent residual zones using steam injection, vapor extraction, resistive heating, and other innovative methods.
 - Funnel-and-gate technology for in situ remediation of contaminated groundwater.
 - Surfactant-enhanced immobilization and removal of chlorinated solvents dissolved in groundwater.
 - Chemical partitioning tracers for locating pure-phase and residual-phase chlorinated solvents in the subsurface.

Detection of Subsurface Contamination

- ◆ Development of miniature laser induced fluorescence (LIF) sensors and unique biosensors for in situ measurement of organics in groundwater.

Subsurface Characterization using CPT

- ◆ Rapid emplacement of pumping and monitoring wells.
- ◆ Remote determination of subsurface geophysical properties (pore-pressure, resistivity).
- ◆ Real-time subsurface stratigraphic profiles.



Air Pollution Characterization and Control System Design

- ◆ Air pollution control systems for volatile organic compounds (VOCs), nitrogen oxides (NOx), and particulates from manufacturing, maintenance, and operations.
- ◆ Laboratory and field measurements of VOCs, NOx, and other toxic air compounds.

...providing environmental technologies for a sustainable future.

Perchlorate Biodegradation Technology: Multiple Applications

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ABSTRACT

A very robust and cost-effective biodegradation process was developed that reduces the perchlorate ion (ClO_4^-) to chloride (Cl^-). Applied Research Associates designed and fabricated a prototype system for the Air Force Research Laboratory's Environmental Technology Development Branch and integrated the system into an existing waste treatment plant at Thiokol Corporation's production facility near Brigham City, Utah. Since 8 December 1997, Thiokol has used this prototype to treat actual wastewater from production and demilitarization operations. This wastewater contains perchlorate, salts, corrosion inhibitors, and other contaminants. The ability of this process to destroy dilute perchlorate in complex waste streams directly contributes to the success of Thiokol's ammonium and potassium perchlorate recovery and reuse operations. This success has also led to many new applications. Treatability studies have been conducted on highly contaminated ground water. These studies showed that perchlorate could be successfully reduced in complex brine wastes that contained many competing ions. Chlorate (ClO_3^-), nitrate (NO_3^-), nitrite (NO_2^-), and chromium (VI), were reduced simultaneously with perchlorate. Treatability studies were also conducted on simulated RDX hydrolysate with promising results. The potential applications of this process to wastewater generated from energetic (hazard class 1.1) propellant and pyrotechnic operations appear unlimited. Another potential application is the destruction of the water-soluble constituents in smoke formulations. A biological process that can destroy the oxidizer salts (ClO_3^-) and soluble organics (sugar, lactose) in smokes will greatly simplify disposal processes.

BACKGROUND

A program to develop biodegradation technology for perchlorate was initiated in 1989 under sponsorship of the United States Air Force. At that time biodegradation was recognized as a viable process to treat dilute AP waste streams and treat contaminated ground water. Attaway and Smith¹ isolated an organism capable of reducing perchlorate and designated the bacterium HAP-1. Laboratory studies were conducted in batch and continuous stirred tank reactors (CSTRs). The process variables that affect perchlorate reduction performance were addressed in these studies. Variables included temperature, pH, nutrient type, nutrient concentration, residence time, and perchlorate ion concentration. Other laboratory efforts culminated in the positive identification of the bacterium responsible for perchlorate reduction as *Wolinella succinogenes*². This identification has enabled us to take advantage of the scientific data and literature on this organism and further optimize the original process. Process enhancements resulted in very stable, predictable perchlorate reduction rates exceeding 0.5 g/l per hour. This paper reports on the results of the prototype demonstration to date and on ground water treatability studies. One patent (5,302,285)³ has been granted on this process. Additional patents are pending.

In 1995, a continuous-flow pilot system capable of treating up to 1000 gallons per day of effluent was designed, fabricated, and successfully tested at Tyndall AFB, Florida. In 1996, optimization studies were accomplished that led to an industrial application. Through a Cooperative Research and Development Agreement (CRDA) with Thiokol Corporation, Defense and Launch Vehicle Division, this process was modified to treat perchlorate wastewater containing salts, corrosion inhibitors, and other contaminants. This prototype biodegradation process was integrated into existing waste treatment processes at Thiokol's production facility near Brigham City, Utah, and is operated by Thiokol under the CRDA. The capacity of this system is over 3000 gallons per day, depending on perchlorate and salt concentration.

PROTOTYPE DEMONSTRATION

This demonstration was conducted in two phases. First, the production-scale transportable system was assembled on an existing test site at Tyndall AFB, Florida, and functional and process evaluations were conducted. Process control and operation were demonstrated with two bioreactors in series and parallel configurations. Upon successful completion of the Tyndall demonstration, the system was disassembled, transported to Thiokol, modified, and reassembled. The validation testing at Thiokol is generating performance and cost data in an integrated industrial waste treatment facility.

Results of the Prototype Demonstration at Tyndall AFB, FL

The redesigned and modified prototype system underwent functional testing at Tyndall AFB, Florida, during the summer of 1997. A schematic of the prototype system is shown in Figure 1.

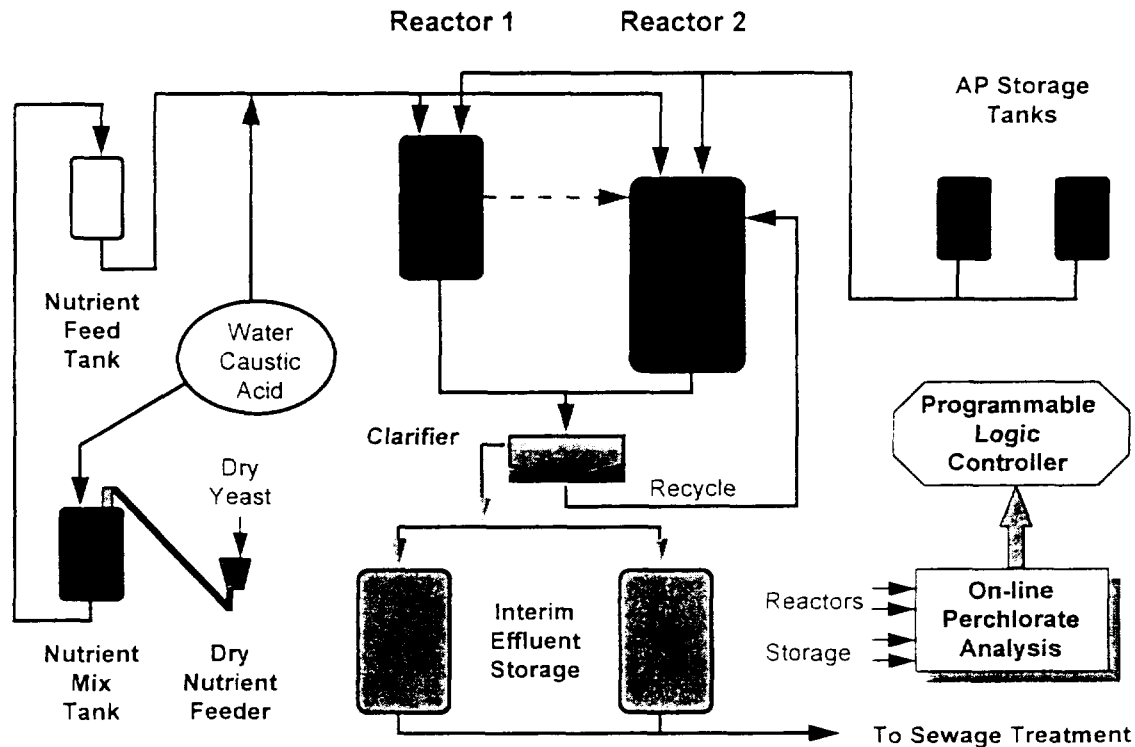


Figure 1. Schematic of the Thiokol Prototype System.

Both parallel and series operations were successfully conducted during the Tyndall demonstration. The inoculum was grown from a lyophilized (freeze-dried) culture, containing *Wolinella succinogenes*, to validate this procedure. A nitrogen generator was used to purge reactor 1 (R-1400) of oxygen to facilitate inoculation. The reactor was charged with approximately 200 gallons of 1000-ppm of ammonium perchlorate solution and nutrient. Temperature and pH were adjusted, and then the inoculum added to the reactor. When the perchlorate concentration began to drop, additional perchlorate and nutrient solution were metered into the reactor. The fill rate of R-1400 was varied to ensure proper inoculation and perchlorate removal. By the time R-1400 was full (720 gallons), the perchlorate was being completely reduced. The effluent from R-1400 was fed to reactor 2, R-1700, along with the additional perchlorate and nutrient necessary to complete the inoculation. The hydraulic volume of R-1700 is approximately 1600 gallons. With both reactors inoculated and accepting feed, parallel operation was initiated. The perchlorate used for the inoculation and start-up was prepared from 10-12% ammonium perchlorate solution from the wash out of Minuteman stage 2 propellant. When both reactors were completely reducing perchlorate, the feed was switched to 10% brine. The composition of the Thiokol brine effluent used in the Tyndall demonstration is provided in Table 1.

Table 1. Composition of Thiokol Brine

Component	Concentration, mg/l	Component	Concentration, mg/l
Perchlorate, ClO_4^-	4266	Chloride, Cl^-	101,160
Nitrate, NO_3^-	4662	Ammonium, NH_4^+	290
Nitrite, NO_2^-	299	Total Dissolved Solids	271,000

Dilution of the brine to 10% was necessary because of the very high total dissolved solids in this effluent. Even a 10% solution resulted in a 2.71% salt content in the reactor before nutrient, acid, and caustic additions. Bench-scale studies showed TDS in the 2-3% range would begin to inhibit perchlorate reduction. After start-up on 100% yeast, the nutrient was switched (7/14/97) to a mixture of cheese whey and yeast (75:25). Figures 2 and 3 show the performance of R-1400 and R-1700 respectively. The spike in the perchlorate concentration in the reactors on 7/19/97 was due to the microbial populations adjusting to the high salt concentration and the new nutrient. In R-1400 the feed was temporarily interrupted for one day to aid in the adjustment. However, in R-1700 the feed was continued despite the upset. Both reactors recovered and performed well during the remainder of the parallel operation.

A short test was conducted with the reactors in series. The nutrient was changed to 100% brewer's yeast, which was fed to both reactors. The effluent feed stream was a concentrated ammonium perchlorate solution fed at a rate to simulate 4000 to 6000-ppm into the reactor. Figure 4 shows that the perchlorate is partially reduced in the first reactor and the reduction is completed in the second reactor. However, at three weeks into the study, the perchlorate feed was further increased to 8000 ppm and the nutrient increased accordingly. The temperature began to increase in the reactors, as seen in Figure 5, and perchlorate reduction performance became erratic. This upset was partially attributed to the high temperature caused by the high solids content and long solids retention time in the reactors. It was previously demonstrated that when reactor temperatures exceed 42°C (108°F), perchlorate reduction is severely inhibited. Operating the clarifier at 100% recycle created the very high solids retention time and overwhelmed the reactors with suspended and dissolved solids. The high recycle rate was acceptable for very dilute effluent/nutrient feeds but not for concentrated effluents. Therefore, the process was modified so that the recycle rate could be controlled to both reactors and to waste.

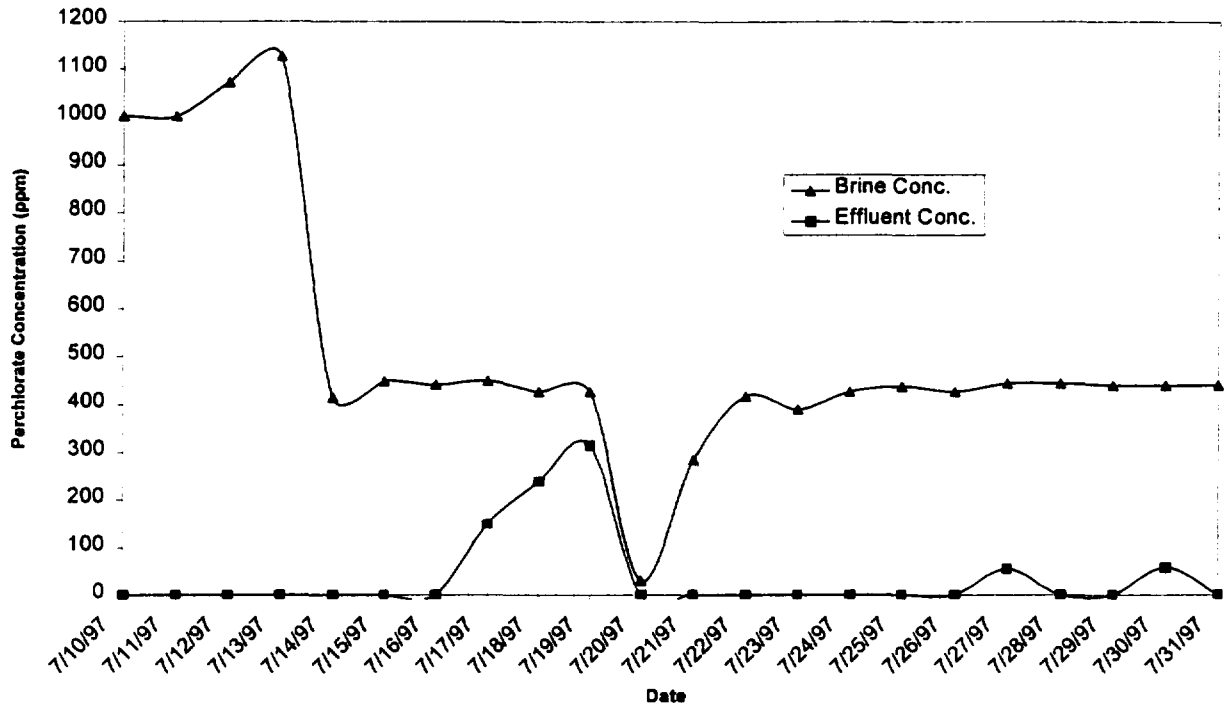


Figure 2. Perchlorate Concentration in R-1400 during Parallel Operation

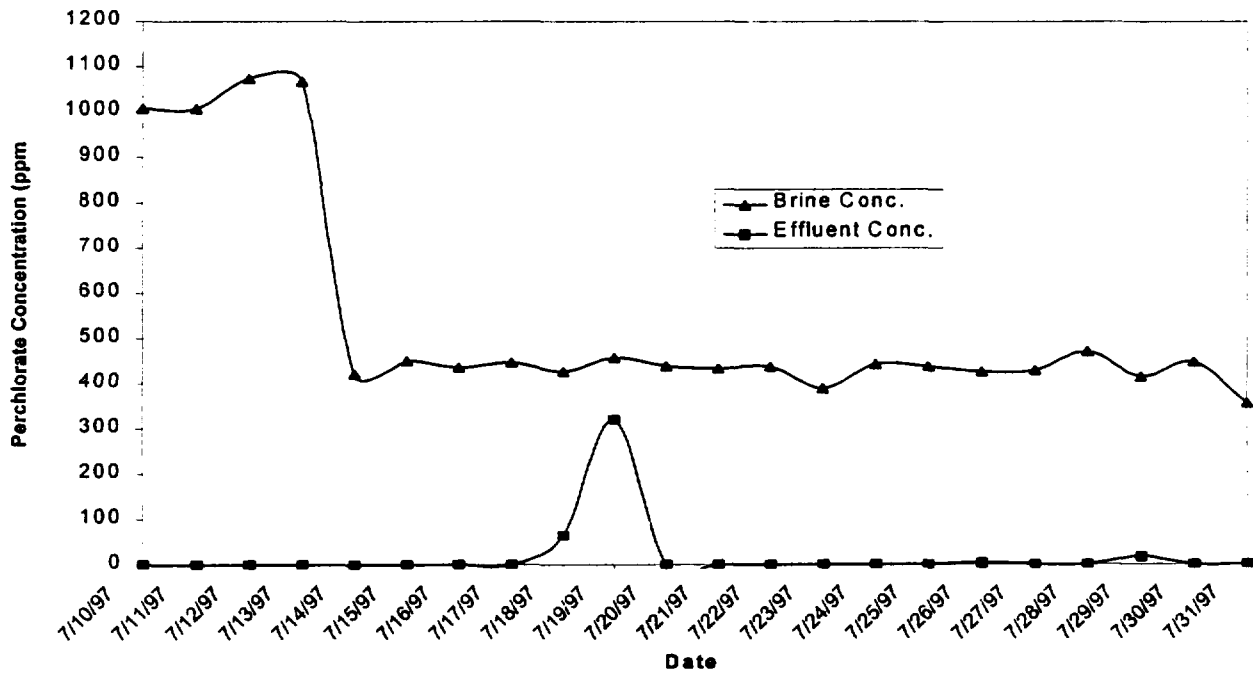


Figure 3. Perchlorate Concentration in R-1700 during Parallel Operation

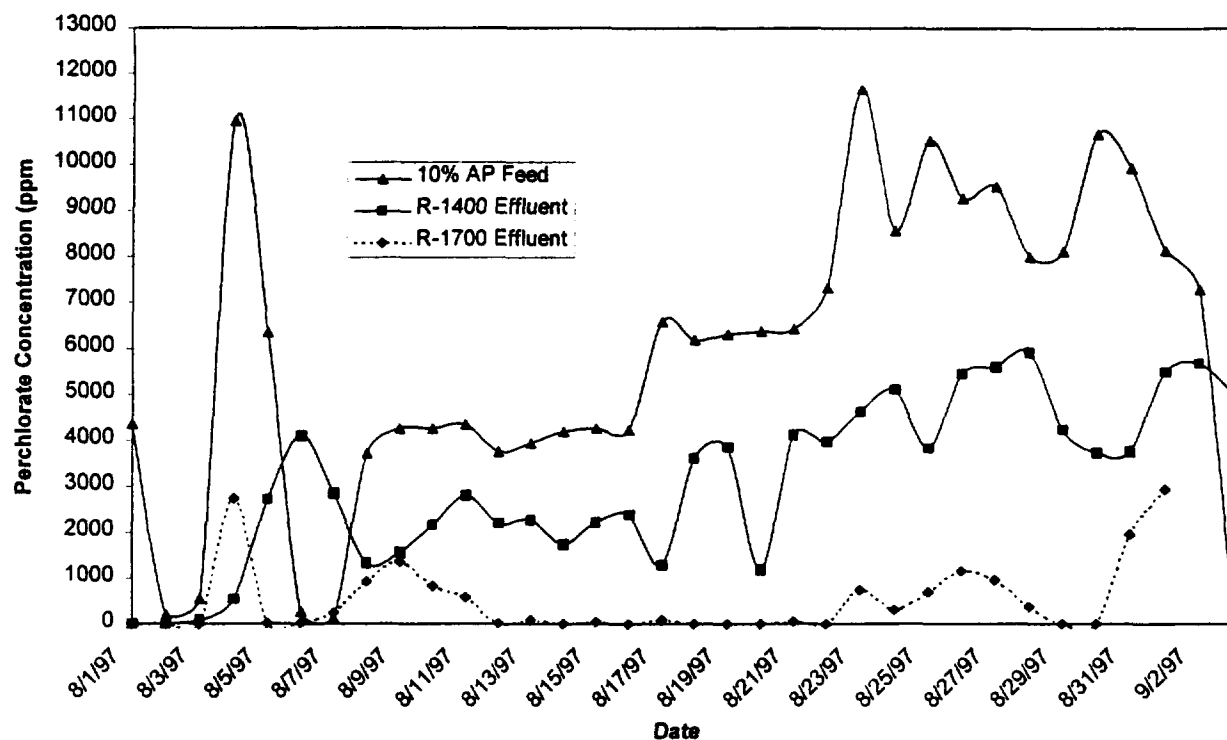


Figure 4. Perchlorate Concentration of the Feed and Reactors during Series Operation

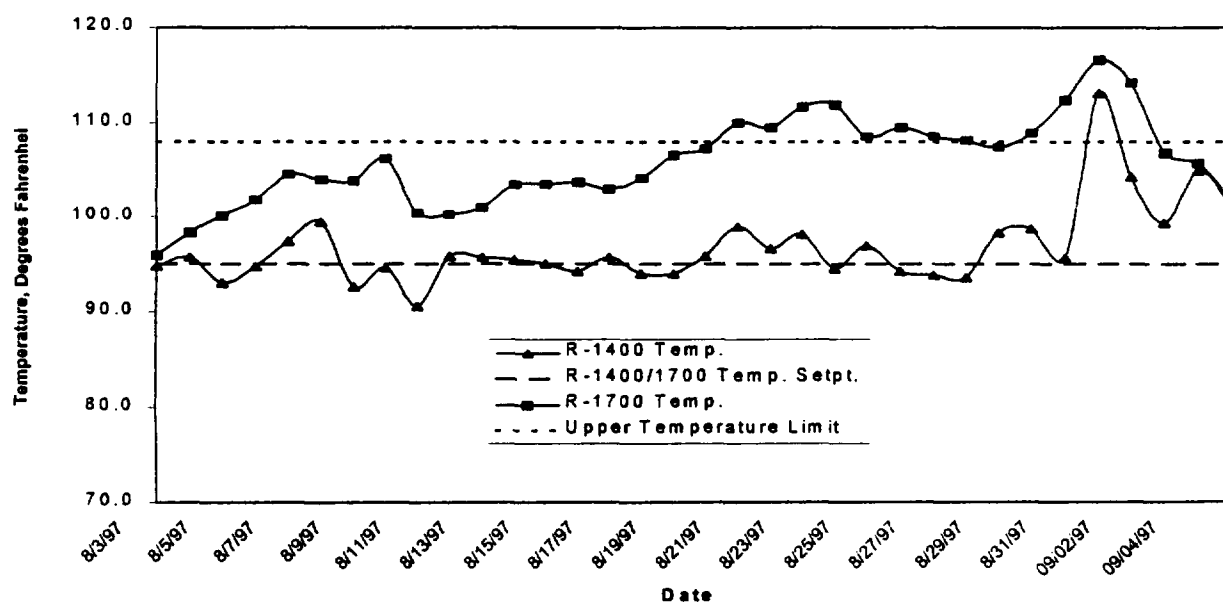


Figure 5. Reactor Temperatures during Series Operation

Prototype Demonstration at Thiokol

Minor process modifications were made during the system installation at Thiokol that began on 13 October 1997. Additional modifications and functional performance tests were conducted in November. On 8 December 1997, the reactors were inoculated using a culture that had been grown from a lyophilized sample. The typical effluent being treated at Thiokol comes from their ion exchange concentration and potassium precipitation units. Therefore, it is very high in TDS (150-300 g/l) and relatively low in perchlorate (~5000 mg/l). However, during the first two months of operation, the perchlorate in the brine effluent was relatively high (20-90,000 mg/l). In addition, the effluent contained approximately 10,000-mg/l nitrite and 5000-mg/l nitrate. The effluent is produced in batch processes; therefore, a different batch was fed to the reactors every 2-8 days. Because of the high TDS, the effluent is diluted to 5-10% of its original concentration as it is fed to the reactor. Figure 6 shows the actual perchlorate concentration in the undiluted feed and in the reactor effluent.

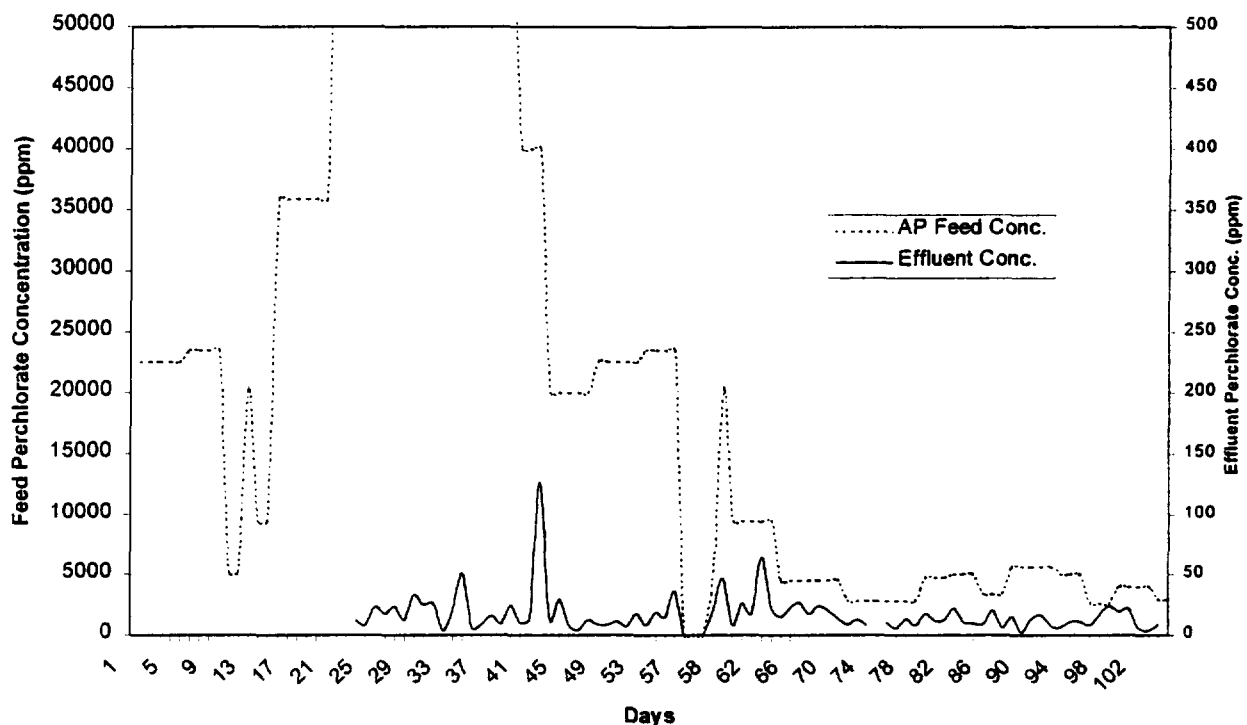


Figure 6. Perchlorate Concentration of Thiokol Brine and Reactor Effluent

During the first four months of operation at Thiokol, the prototype has performed very well. Some process control and software problems prevented optimal performance, nevertheless, perchlorate reduction has been acceptable. Typical perchlorate concentration in the reactor is less than 20 ppm by the ion-specific probe method. These results almost always translate to near non-detect by ion chromatography. The nitrate was also nearly completely reduced, however, little nitrite reduction was observed. During this four month period there has not been a major upset in operation and the re-inoculation has not been necessary.

PERCHLORATE REDUCTION IN WATER FROM THE MUDDY CREEK AQUIFER

The discovery of perchlorate in the Colorado River led to the identification of a source area near Henderson, Nevada. Ammonium perchlorate has been manufactured in this area since the 1950s. Perchlorate in the soil and groundwater has contaminated shallow aquifers that feed the Las Vegas Wash, which flows into Lake Mead and the Colorado River. Water from one test well in this aquifer had a perchlorate concentration of 3700-ppm, which is over 200,000 times the 18-ppb action level established by the California Department of Health Services (DHS). Lake Mead supplies drinking water to over 12 million people in southern Nevada and California. Perchlorate concentration in Lake Mead, and in the drinking water from Lake Mead, has been measured near the 18-ppb action level established by the California DHS. Therefore, this is an acute problem and potential excellent application for perchlorate biodegradation.

Currently, some of the water in this aquifer is being pumped to the surface and treated to reduce chrome (VI). This water, which contains perchlorate and other contaminants, is then injected back into the aquifer. Biodegradation of the perchlorate in this water before it is re-injected could mitigate this acute problem. The nominal composition of water from the aquifer is provided in Table 2.

Table 2. Nominal Composition of Water from the Muddy Creek Aquifer

Component	Concentration, mg/l	Component	Concentration, mg/l
Perchlorate, ClO_4^-	1200-1500	Calcium, Ca^{+2}	800
Chlorate, ClO_3^-	3000-3500	Magnesium, Mg^{+2}	400
Sulfate, SO_4^-	1700	Nitrate, NO_3^-	200
Chloride, Cl^-	2000	Boron	14
Sodium, Na^+	1800	Chrome (VI)	9

Treatability studies were conducted on this water in laboratory-scale CSTRs. Since the total dissolved solids of this water was less than 2% (~120 g/l), the water was mixed with nutrient and fed directly to the reactors. However, water from this aquifer contained high concentrations of contaminants that had not been previously evaluated in this perchlorate biodegradation process. Two contaminants of particular concern were chlorate (ClO_3^-) and chrome (VI). Studies were designed to determine if contaminants would compete with, or inhibit, perchlorate reduction. Specific objectives were to:

- Determine if perchlorate is biological reduced in this ground water matrix
- Determine the fate of the chrome (VI)
- Determine the impact of chlorate (ClO_3^-) on perchlorate reduction
- Determine the effect of indigenous microbes on perchlorate reduction

The results of over 7000 hours of testing were very positive. Long-term studies were conducted in different reactor configurations using both typical and alternate nutrients to minimize nutrient consumption and cost. Both chlorate and perchlorate were effectively reduced to chloride. Typically all the chlorate was reduced before perchlorate could be completely reduced. However, chlorate reduction consumed little additional nutrient. Perchlorate was completely reduced with 0.05 pounds of nutrient per gallon of effluent. This translated into \$0.015 per gallon for the nutrient mixtures evaluated. Preliminary tests using milk and brewery process wastes were also very promising and could further reduce the nutrient cost to less than a cent per gallon.

Chrome did not inhibit perchlorate reduction and was reduced to chrome (III) in the process. Chrome (VI) was reduced from 8-9 ppm to less than 0.2 ppm. Nitrate was also simultaneously reduced. One test was specifically designed to evaluate the effect of the indigenous microbes that were present. Screening tests on these microbes showed they could not reduce perchlorate. A reactor was operated for over two months with a "neat" feed stream of un-sterilized ground water containing these microbes. Perchlorate reduction performance did not deteriorate during this period.

REDUCTION OF PERCHLORATE IN HYDROLYSATE

An effective and accepted way to destroy the energetic character of propellant and pyrotechnic ingredients in water is through base hydrolysis. Significant research has been conducted in this area and several demonstrations and operational processes currently exist. Some propellants, explosives and pyrotechnics (PEP) contain perchlorate. If perchlorate can be reduced in hydrolysates of PEPs and other hydrolyzed process waste streams, then more cost-effective waste disposal options are possible. To test the feasibility of this concept, a surrogate of RDX hydrolysate containing perchlorate was evaluated. The surrogate was based on experimentally determined complete hydrolysis products of RDX⁴. The composition of the actual feed material prepared for this test is provided in Table 3. The amount of RDX required for this formulation equated to 2.22 grams per liter. Theoretically, the concentration of this surrogate could be increased several fold. During these tests perchlorate was completely reduced. In addition, some of the hydrolysis products, particularly formate, were consumed as nutrients.

Table 3. Composition of Surrogate RDX Hydrolysis Containing Perchlorate

Component	Concentration, mg/l	Component	Concentration, mg/l
Perchlorate, ClO ₄ -	~1000	Formaldehyde, CH ₂ O	330
Formate, CHO ₂ -	675	Nitrite, NO ₂ -	236
Acetate, CH ₃ O ₂ -	220	TDS	~3200

REDUCTION OF CHLORATE IN DYE COLORED SMOKES

Dye colored smoke formulations typically contain fuel, oxidizer, and dye components. Chlorate (ClO₃-) salts are the oxidizers and dextrose or similar materials make up the fuel and binder components. Biodegradation of the water-soluble components of smoke formulation could greatly improve disposal options. For instance, hydrothermal oxidation (HTO) has been considered for smoke disposal. A major technical hurdle of this technology has been salt and acid-handling problems caused by the high chlorate concentrations. The treatability studies conducted on water from the Muddy Creek Aquifer demonstrated that chlorate can be effectively degraded by the same process that degrades perchlorate. In addition, water-soluble fuel components may provide the nutrient required to drive the biodegradation process. Destruction of chlorate and other water-soluble components in this manner would greatly simplify the hydrothermal destruction of the dye components, increase process rates, improve reliability, and potentially reduce cost.

CONCLUSION

The process described in this paper is a proven, low-cost approach to addressing the many perchlorate treatment needs brought about by more stringent and widespread environmental regulation. Disposal of ammonium perchlorate from production, remanufacturing, test and evaluation, and remediation activities is an industry-wide, national problem. The demonstrated ability to effectively biodegrade dilute ammonium perchlorate wastes enables continued use of this critical defense material in both rocket motors and ordnance items. In addition, it has been demonstrated that nitrate, nitrite, chlorate, and chrome VI can be reduced with, or without, perchlorates present. Highly contaminated ground water has been effectively treated to reduce mixtures of the above contaminants to acceptable discharge levels. The use of low-cost, alternate nutrients, and even process waste materials, has reduced nutrient cost to near a cent per gallon or less and makes this a very cost-effective process to implement and operate. The ability to destroy perchlorate to non-detect levels in complex waste streams is a distinct advantage over selective concentration and catalytic destruction processes that can become fouled or overwhelmed by co-contaminants. Over eight years of research and development has resulted in a mature process that can be applied to many environmental problems related to perchlorate, nitrate, and mixed energetic wastes.

REFERENCES

1. Attaway, H., and Smith, M. 1993. "Reduction of Perchlorate by an Anaerobic Enrichment Culture." *Journal of Industrial Microbiology*. 12: 408-412
2. Wallace, W., Ward, T., Breen, A., Attaway, H. 1996. "Identification of an Anaerobic Bacterium Which Reduces Perchlorate and Chlorate as *Wolinella succinogenes*." *Journal of Industrial Microbiology*. 16: 68-72
3. United States Patent 5,302,285. "Propellant Wastewater Treatment Process." April 12, 1994
4. Heilmann, H., Wisemann, U., Stenstrom, M., 1996. "Kinetics of Alkaline Hydrolysis of High Explosives RDX and HMX in Aqueous Solution and Adsorbed to Activated Carbon." *Environmental Science & Technology*. 30: 1485-1492